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Art. VI.—*Two Gregarines from Ctenolepisma Longicaudata with Notes on Forms in other Silverfish.*

By EDER LINDSAY, B.Agr.Sc.

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During the study of the feeding habits of the silverfish *Ctenolepisma longicaudata* Esch. it was found that these insects were frequently infected with gregarines. This silverfish has not been studied previously, but gregarines have been found in related genera. Watson-Kanm recorded one gregarine from *Lepisma saccharina, Gregarina lagenoides* Labbé, but did not mention the two gregarines which J. W. Cornwall, 1915, had found in the same species. More recently J. A. Adams, 1935, described two new forms from *Thermobia domestica,* namely *Lepismatophila thermobiae.* and *Colepismatophila watsonae,* for which he created the two new genera.

Two new gregarines were found in the mid-intestines of *Ctenolepisma longicaudata,* and these have been named *Lepismatophila ctenolepismae,* sp. nov. and *Gregarina ctenolepismae,* sp. nov. The former is a large form which was easily seen during the dissections, but the latter was noticed only when the insects were examined under the binocular ($\times 70$).

Most of the insects were collected in Melbourne, but the wide distribution of the parasites was evident from the infested insects collected in Toowoomba and Brisbane, Queensland; Bigga, N.S.W.; Renmark, S.A.; Launceston, Tasmania; and Boyup Brook, W.A.

Materials and Methods.

As the trophozoites live in the mid-intestine, the alimentary canals posterior to the crop were disseeted out, slit longitudinally, and the contents examined in water. Fixed preparations were also made following the methods of MacKinnon and Ray 1933. The alimentary canals were immersed in Carnoy's fluid for twelve hours. Some were then stained twenty minutes in Ehrlich's haematoxylin and finally dissected in clove oil. Others were cut into sections 5-7μ thick, longitudinally and transversely, and stained three minutes in Ehrlich's haematoxylin. This stain differentiated clearly the different parts of the protoplasm of the gregarines. The trophozoites were more difficult to fix than the tissues of the gut, and in several sections the larger ones tended to break.

The cyst wall was impervious to stains and no attempt was made to follow the nuclear changes associated with the development of the cyst, but at various stages during the ripening the contents of the cysts were examined after fixing with Carnoy, breaking the wall, and staining with haematoxylin or saffranin.

In the dissected insects the trophozoites of both forms were found attached to the epithelium of the crypts in the sacculi and anterior part of the mid-intestine. Here they caused the atrophy of the neighbouring cells, but apparently no other damage, even where the infestation was so heavy that the parasites occupied the greater part of the gut. The infestations of both forms were usually heavy, frequently 20 or 30 individuals of both gregarines occurring in a single insect. Generally these were of various sizes, and observations extending over two years showed that the gregarines had no seasonal cycle so that the silverfish were always liable to be infested.

Lepismatophila ctenolepismae sp. nov.

This form was found in about 80 per cent. of the silverfish examined.

Trophozoite.—The earliest stages of the life cycle are presumably passed inside the epithelial cells, but the smallest trophozoites found were already extracellular. In one case a minute form was located, shown in Fig. 3, which may be a young trophozite. The body of the smallest form which could be recognized with certainty was cylindrical though not divided, and had a large spherical epimerite embedded between the epithelial cells. Observations at later stages showed that the septum between the protomerite and deutomerite then develops, and the front end becomes broader and broader, until the whole trophozoite becomes roughly "turnip-shaped" (Figs. 4 and 5). Later the more mature trophozoite rounds off with the protomerite remaining as a capping at one end. It was of interest to notice that the "tail" region gradually merged into the body of the trophozoite without leaving the sucker-like scar referred to by MacKinnon and Ray, 1931, in *Selenidium*.

The protoplasms of the three divisions of the young trophozoite appear different in transmitted light and stain differently with Ehrlich's haematoxylin. That of the deutomerite is the most densely granular particularly around the nucleus. That of the protomerite, though less dense, shows a coarse structure when fixed and stains more deeply than the deutomerite. This same coarse structure appears, however, in the deutomerites of many of the more mature trophozoites (Fig. 2, *l.c.*), and frequently tiny granules which stained deeply with haematoxylin were found imbedded in the protoplasm. The protoplasm of the epimerite is densely granular and when fixed shows a fine structure.

The nucleus is 25–40μ in diameter in about the centre of the wide part of the deutomerite. By exerting gentle pressure it can be moved about the body. When stained it shows one caryosome.

The body is bounded by the pellicle which is thin in the region of the epimerite and protomerite but forms a thick refractive coat to the deutomerite and, in the tail region, is thrown into three or four transverse ridges. In the transverse section the "tail" shows as a lightly granular disc surrounded by more deeply stained material enclosed by the thick pellicle (Fig. 2*a*). The mature trophozoites break free of the intestinal walls, usually with the epimerites still attached. These latter become less granular as the gregarines move down the intestine, and finally disappear from the trophozoites. Detached epimerites were never found.

Some of the free trophozoites still remain pressed close to the walls below the sacculi, fitting into small depressions where the epithelial cells have atrophied (Fig. 2). The mature trophozoites pass down the mid-intestine in groups of 20 or 30 lying between the intestinal wall and the peritrophic membrane (Fig. 19, Plate VI.). No temporary associations are made in the group, but when the trophozoites reach the posterior end of the mid-intestine association occurs, protomerite to protomerite, usually between only one or two pairs at a time. These sporonts secrete a white cyst wall, and pass into the hind intestine. Unassociated sporonts did not pass into the hind intestine as had been noticed by Cornwall.

Cyst.—The shining white cysts are conspicuous in the faecal material, usually one being attached to each pellet. They are of two distinct forms, round and oval. As both these forms gave rise to the same kind of spores they were considered to belong to the same species of gregarine. Of 25 cysts measured, the average size of the spherical cysts was $252\mu \times 238\mu$ and of the larger oval cysts $316\mu \times 466\mu$ (standard deviation of 14μ).

The cysts were freed of faecal matter and kept under both moist and dry conditions at 23°C. so that the development could be followed. The cysts from fresh faeces usually contain two sporonts although development sometimes proceeded further inside the intestine. Development proceeds under dry conditions or in water. The cyst wall darkens, becoming first a mottled grey, then black as the cyst ripens. The sporonts divide into many gametes, each about 13μ diameter, but no sexual differentiation could be seen. Then hundreds of tiny spores, almost transparent, separate around the granular residual protoplasm which forms into many non-nucleated masses, which become more definite, though they still can be readily plasmolysed. These and the large oil globules in the cyst gradually disappear as the spores ripen. The spores have a large central nucleus and thick transparent walls which gradually become thicker and darker. At the same time the cyst wall becomes darker, and

the pitted and grooved dark outer layer can be separated from the colourless inner layer (Fig. 8). The cyst finally ripens in about fourteen days.

The ripe cyst is black and dented, and through the semi-transparent wall the dark spores can be seen crowded towards one end. The ripe spores are black, semi-transparent, flattened ovals with one side more curved than the other, 15 to 17μ long, 4 to 6μ wide, and 4μ thick. Each has a large globule at its centre with smaller globules at the end and along the more curved side (Fig. 9). The spores tend to hold together end to end (Fig. 10) and when liberated by the rupture of the cyst come out in many long spring-like chains, extending in some cases for about 8 mms. around the cyst.

The spores contaminate the food and so reach the insects' intestine again. Many spores were found in the crops examined. Some were broken across the middle and some at one end; but no spores split longitudinally as described by Cornwall. Broken spores were also found in the hind intestine but no spores were noticed in the act of germination. The spores could not be broken by pressure and stains would not penetrate the thick capsule so that the sporozoites could not be distinguished. But the heavy infestations would suggest that each spore contains several sporozoites. (Cornwall found 8 sporozoites.)

TABLE 1.—MEASUREMENTS OF TROPHOZOITES OF LEPISMATOPHILA CTENOLEPISMAE (MICRONS).

Total Length.	Protomerite.		Deutomerite.		Epimerite Width.
	Length.	Width.	Length.	Width.	
429	352	168	76	153	..
400	..	..	..	184	..
386	306	177	79	165	..
341	262	81	..	..	37
283	..	171	..	153	..

GREGARINA CTENOLEPISMAE sp. nov.

This smaller gregarine was found in about half the silverfish dissected.

Trophozoite.—The trophoziote is heart shaped, about as long as wide, and attached to the wall by a peg-like epimerite set in the depression at the top of the "heart." The body is not divided into protomerite and deutomerite. The nucleus (6–9μ diameter) is at the base of the depression, and seems to be attached to the pellicle (cf. Chakravarty, 1935). In the living gregarine it is hidden by the densely granular protoplasm. When fixed, the protoplasm shows a finer structure than in the previous form. Around the nucleus it stains deeply with haematoxylin, and deeply stained strands radiate from the nuclear area. The pellicle is thin.

The smallest form recognized measured $5\mu \times 7.3\mu$ (Fig. 13), but most of the gregarines were larger than this (Table 2). The gregarines occur singly or in pairs, the outer one, the satellite, fitting over the primite still attached to the wall (Figs. 11 and 12). There seems to be no other record of syzygy occurring while the gregarines are still attached to the walls (Wenyon 1926, p. 1146, and Henry 1932). Usually both members are the same size, or the satellite is a little larger. During dissection the larger pairs readily come free of the wall with the epimerite still attached. They frequently separate from each other and roll over so that the epimerite is hidden in the depression at the top.

Usually only a few free pairs of trophozoites and associating sporonts are found in the lower part of the mid-intestine, instead of the large group of unassociated trophozoites found in the *L. ctenolepismae* infestation. It is not certain that the same pair remained associated until mature, for by the time the sporonts had encysted they were no longer fitting over each other but were in the position indicated in Fig. 14.

Cyst.—The small shining white cysts containing the two sporonts pass out with the faeces, usually three or four adhering to each pellet. At first the wall is thin (3μ), but thickens to about 6μ and finally to 10μ as the sporonts fuse and the cyst ripens. It stains deeply with haematoxylin. In three days eight to nine spore ducts protrude through the cyst wall from the protoplasmic mass inside. These grow out about 10μ from the cyst (Figs. 15 and 16). In six days at 23°C. the tiny white

TABLE 2.—MEASUREMENTS OF GREGARINA CTENOLEPISMAE (MICRONS).

Unassociated Trophozoites.	
Width.	Length.
18·0	18·0
34·0	31·6
73·4	65·5
78·6	78·6
78·6	76·0
92·0	82·0
94·3	91·8
99·8	94·3
145·0	141·0
161·0	122·0

Trophozoites in Syzygy.		Young Cysts.	
Width.	Total Length of Pair.	Width.	Length.
microns	microns	microns	microns
84	145	69	69
107	153	107	95
153	184	84	69
230	260	100	107
..	..	84	84

refractive spores exude in chains from the ducts like fine white filaments extending about 1.4 mm. around the cyst (Fig. 17). The regular oval spores measure $3.2\mu \times 2.2\mu$. The small amount of residual protoplasm consists mainly of globules about half the size of the spores.

The cyst matures in the dry faeces. If kept in water the young cysts plasmolyse, and the older cysts swell till about 125μ diameter.

Feeding Experiments.

An attempt was made to follow the development of the gregarines in the intestine of the silverfish. The insects were supplied with clean food until cysts were no longer found in the fresh faeces. They were then fed for two days with food artificially infected with ripe spores of both gregarines, and then removed to clean food which was changed daily. Subsequent dissections and sections revealed trophozoites of various ages in the mid-intestine and many spores in the crop, but no germinating spores or very small trophozoites. The insects were held at 23° C. throughout.

To find the duration of the trophozoite stage a group of naturally infested insects were fed on clean food and the faeces examined daily for cysts. Dissection of several insects, during these observations revealed the presence of trophozoites in the mid-intestine. After 26 days, however, no more trophozoites of *Gregarina ctenolepismae* were present and cysts were no longer found in the faeces. Further, on the 35th day no cysts of *Lepismatophila ctenolepismae* were found, and dissection of the remaining thirteen insects showed that only one harboured mature trophozoites. Since sporonts and cysts were never found to collect in the hind part of the intestine it is concluded that these periods give a lower limit to the duration of the trophozoite stage.

To facilitate the examination of the mid-intestine the infected food was stained with Sudan III. which is taken up by the epithelial cells. The trophozoites of *Gregarina ctenolepismae* take up a little of the red and appear pink surrounded by the red epithelial cells. The trophozoites of *Lepismatophila ctenolepismae* remain uncoloured.

Systematic Position.

In general characteristics the two gregarines described resemble somewhat those found by Cornwall and Adams, but detailed examination showed several differences. For example, *Lepismatophila ctenolepismae*, sp. nov. resembles *Lepismatophila thermobiae* but the spores have not the regular oval shape described by Adams, one side being more convex than the other.

It also resembles Cornwall's form A, but the spore walls are smooth instead of pitted. *Gregarina ctenolepismae*, sp. nov. resembles Cornwall's form B, but the epimerite is peg-like rather than acicular as figured by Cornwall.

Following the classification of Watson-Kamm (1922) both gregarines are placed in the family Gregarinidae; the larger form as a new species of Adams' genus *Lepismatophila*, *Lepismatophila ctenolepismae*, and the small form as a new species of *Gregarina* (Dufour), *Gregarina ctenolepismae*.

Diagnosis.

Lepismatophila ctenolepismae sp. nov.

No syzygy in sporonts. Trophozoite septate, conoid, 390μ $\times$ 164μ. Epimerite smooth, globular. Cyst round 245μ diameter; or oval, 315μ $\times$ 460, dehisce by rupture. Cyst wall black, pitted and grooved. Spores in uncoiling chains. Spores black, ellipsoidal, one side more convex than the other, 16μ $\times$ 5μ $\times$ 4μ. Spore wall smooth.

Gregarina ctenolepismae sp. nov.

Syzygy in cephalonts and sporonts. Trophozoites heart shaped 87μ wide $\times$ 80μ long, non-septate. Epimerite simple peg shape. Cyst white, smooth-walled, spherical 85μ diameter. Spores liberated in chains through spore ducts. Spores oval 3.2μ $\times$ 2.2μ. Spores white smooth walled.

Gregarines in other Silverfish.

The intestines of other species of silverfish were examined for gregarines. *Lepisma saccharina*, the common pest of the Northern Hemisphere is found only occasionally in Australia. Of the six so far examined, three harboured a trophozoite, (Fig. 18) different from the gregarines so far recorded for the *Lepismatidae*. *Ctenolepisma lineata* var *pilifera* is another form collected occasionally with the common silverfish. Three individuals were examined, and in one were found several trophozoites resembling *Lepismatophila ctenolepismae* Luc. These insects had been kept for a time in captivity and several cysts were found in the faeces which had collected. These were small and round and contained the typical spores of this same gregarine. Somewhat similar trophozoites were found in two preserved specimens of *Thermobia aegyptica* Luc. A few native silverfish have been examined, one *Acrotelsella*, and one *Heterolepisma*, collected with the common silverfish, two from termites nests, and two from under bark, but none have contained gregarines.

It is interesting to note that the three species collected together in houses have not the same parasites though they have the same feeding habits and presumably would have the same chances of infection. *Lepismatophila ctenolepismae* occurs only in *Ctenolepisma longicaudata,* and *Ctenolipisma lineata* var *pilifera* which have sacculi in the mid-intestine and not in *Lepisma saccharina* where these are absent. Sufficient material of *Thermobia aegyptica* was not available to permit any conclusion regarding the identity of the gregarines found, and further examination is necessary before any importance can be attached to the absence of gregarines in the native forms.

Summary.

Two new gregarines have been found in the silverfish *Ctenolepisma longicaudata* Esch—which have been named *Lepismatophila ctenolepismae* and *Gregarina ctenolepismae.* These have been described and their life cycle followed. Further, to determine the specificity of the gregarines, several other species of silverfish have been examined. *Lepismatophila ctenolepismae* sp. nov. was found only in *Ctenolepisma lineata* var. *pilifera* Luc. A different form was found in *Lepisma saccharina* (Fig 18) and two unidentified forms in *Thermobia aegyptica* Luc.

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Explanation of Figures.

Fig. 1. Longitudinal section of the anterior portion of the mid-intestine showing trophozoite stage of the two gregarines, l.c. *Lepismatophila ctenolepismae,* and g.c. *Gregarina ctenolepismae,* in the epithelial lining of the sacculi. Portions *a* and *b* show the sacculi not cut in true section, *f* folds in epithelium below the sacculi, *crp.* crypts, *v.* villi. × 38.

Fig. 2.—Tangential longitudinal section of the lower mid-intestine, showing a group of mature trophozoites. *l.c. L. ctenolepismae, a* section of "tail" region, *d.pr.* sporonts with dense protoplasm, *g.c. G. ctenolepismae.* × 38.

Lepismatophila ctenolepismae.—Figs. 3-10.

Fig. 3.—A form found in the sections which may be a young trophozoite 9 μ × 2 μ. × 250.

Fig. 4.—Young trophozoite showing epimerite, drawn from two successive sections. × 250.

Fig. 5.—Outlines of both free and attached forms of trophozoites drawn from living material to show the variation in shape. × 100.

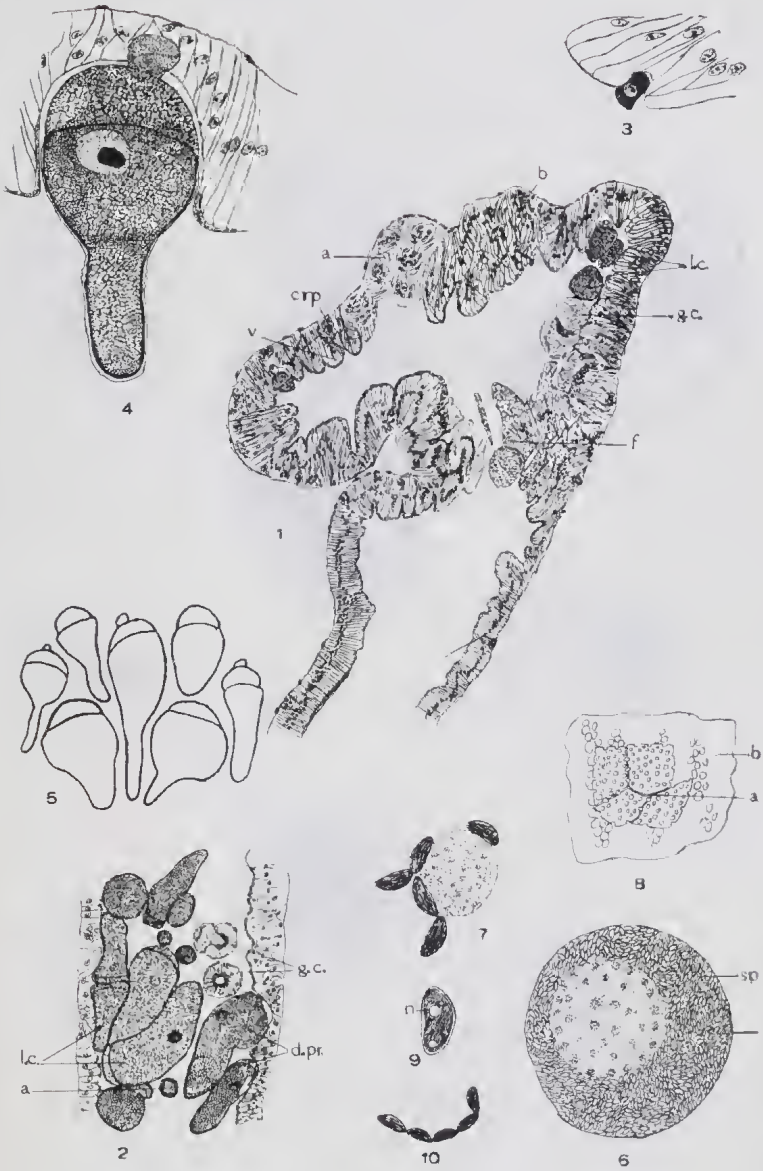
Fig. 6.—Optical section of developing cyst. The transparent spores have separated around the central mass of protoplasm. × 60.

Fig. 7.—Young spores and spherical mass of protoplasm from a fractured cyst. × 300.

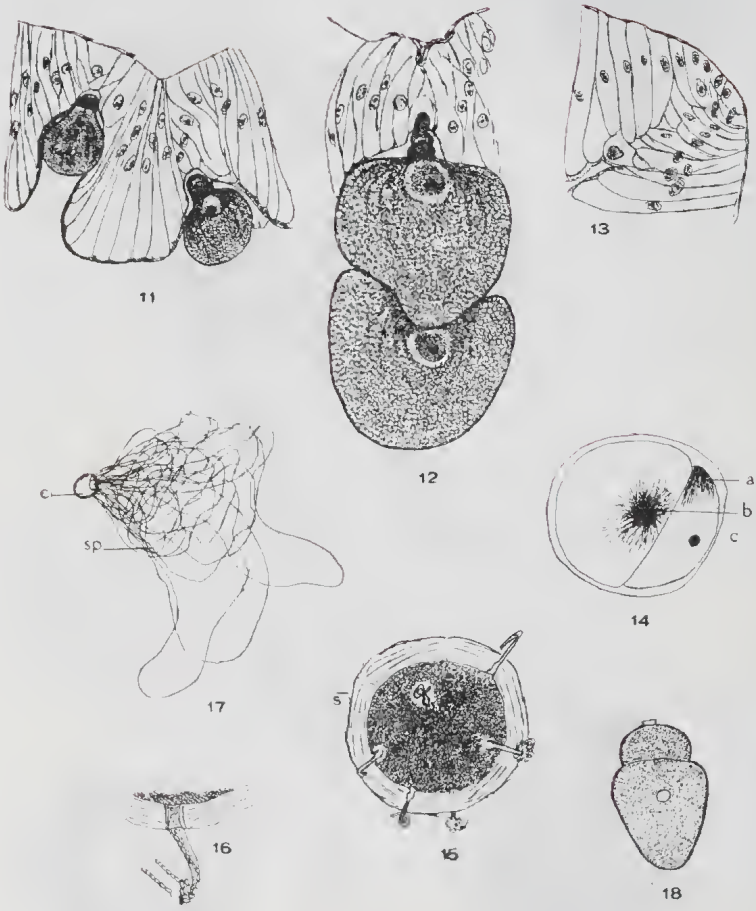
Fig. 8.—Cyst wall with pits and grooves.
a. Groove in outer black layer of wall.
b. Inner colourless layer marked by circular areas. × 340.

Fig. 9. Optical section of ripe spore, showing the nucleus *n* and other globules. × 310.

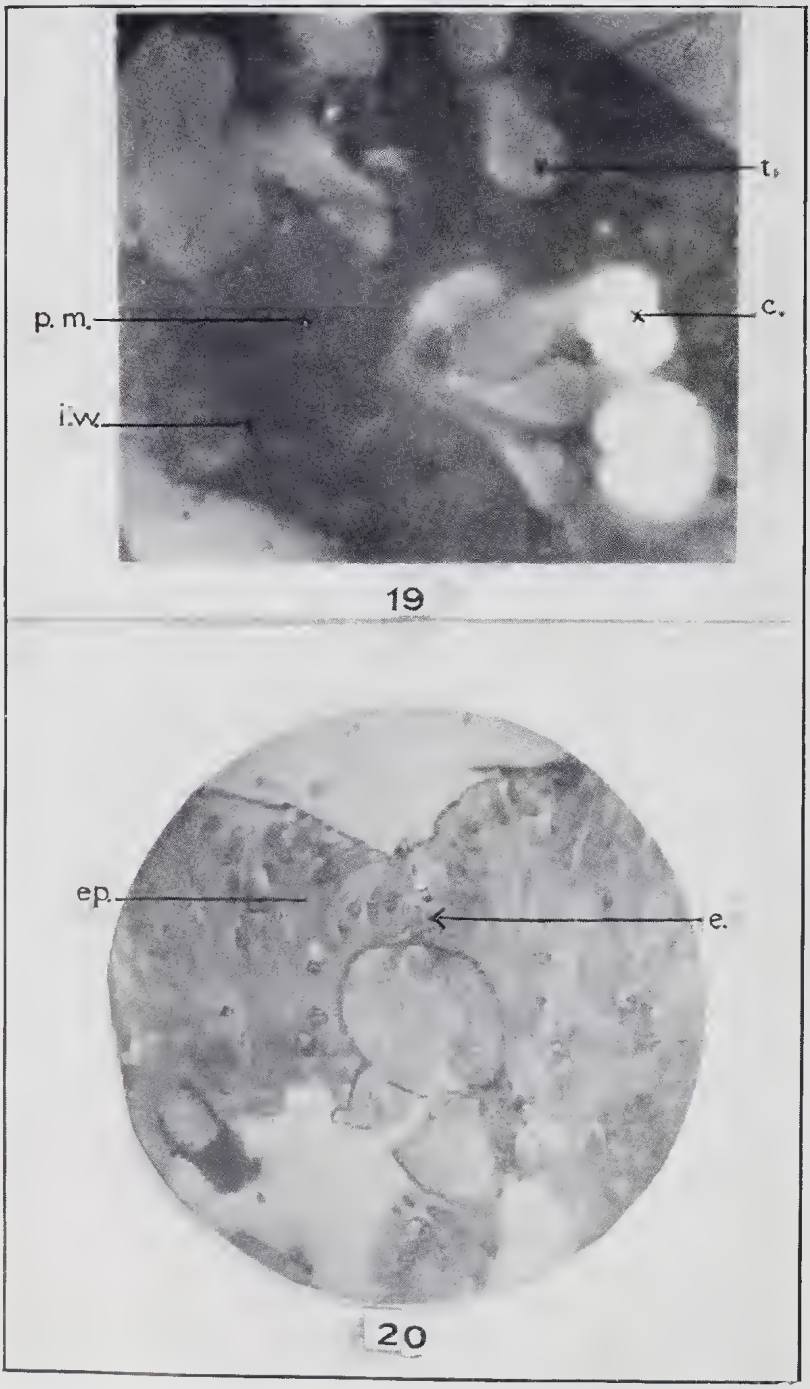
Fig. 10. Chain of ripe spores. × 330.



Text Figs. 1–10.



Text Figs. 11–20.



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GREGARINA CTENOLEPISMAE.—Figs. 11-17.

Fig. 11.—Trophozoites. ×250.

Fig. 12.—Large trophozoites in syzygy drawn from 3 sections. × 250.

Fig. 13.—A small gregarine intracellular 4.9μ × 7.3μ (× 250).

Fig. 14.—Optical section of a newly formed cyst enclosing the associated sporonts. Stained with Ehrlich's haematoxylin.

a. Heavily stained area of second sporont. × 340.

b. Heavily stained protoplasm surrounding the nucleus.

c. Nucleus of second sporont.

Fig. 15.—Cyst showing spore ducts. × 340.

Fig. 16.—Spore dust of cyst with chain of spores exuding through duct. × 400.

Fig. 17.—Ripe cyst *c*. with chains of spores *sp*. exuded. × 15.

Fig. 18.—Unidentified trophozoite from *Lepisma saccharina*. × 180.

PLATE VI.

Fig. 19.—Microphotograph lower part of mid-intestine opened to show trophozoites *t*. and cysts *c*. of *Lepismatophila ctenolepismae* lying between the intestinal wall *i.w*. and the food enclosed in the peritrophic membrane *p.m*. × 480.

Fig. 20.—Microphotograph of large trophozoites of *Gregarina ctenolepismae* in syzygy, attached to the epithelium *e.p*. by the epimerite *e*. × 230.

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